Blaney, W. M. Birkbeck College, London, England. Some observations on the sperm tail of D. melanogaster.

This investigation was prompted by the observation (Oster, Duffy and Binnard, 1966) that the sperm tail of D. melanogaster consisted of two distinct, and in some circumstances separable, filamentous units. It was felt that electron microscopic study ought to clarify this suggestion.

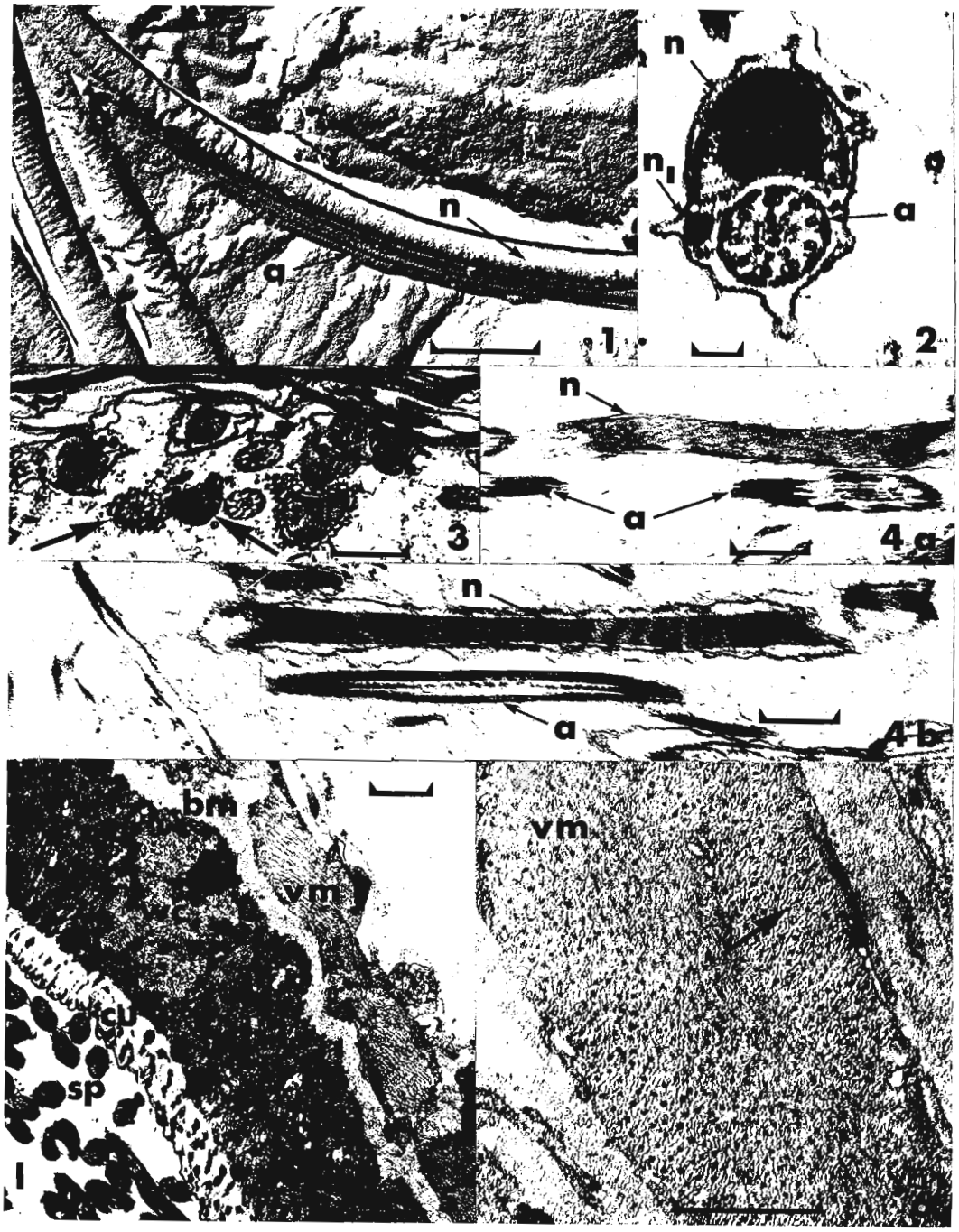
D. melanogaster was used and old females which had been with males were chosen so as to increase the likelihood of sperm being present in the reproductive system.

Observations were made on shadowed carbon replicas of individual sperm tails and on ultrathin sections of ventral receptacle and spermatheca using a Zeiss E.M.9 electron microscope. For carbon replication the ventral receptacle was dissected out on a glass slide in a drop of Drosophila Ringer's solution, with slide and instruments coated with 'Silicone Repelcote' to minimise surface tension problems. The organ was then transferred to a fresh micro-drop of Ringer's solution and gently teased apart to release the contained sperm. Fixation was effected by bringing a drop of 1% osmium tetroxide solution as close as possible to the drop of Ringer's solution containing the sperm and holding it there for one minute. The sperm were then picked up on copper grids coated with a formvar film, and allowed to air-dry. The grids were then washed carefully in distilled water to remove the precipitated salts of the Ringer's solution and any proteinaceous material derived from the rupture of the ventral receptacle. Carbon replicas were then made (Pease 1964) and shadowed with gold/paladium alloy. For preparation of thin sections, the ventral receptacle and spermatheca were fixed in buffered 2.5% glutaraldehyde and post fixed in buffered 1% osmium tetroxide, both adjusted with sucrose to give 0.25 M solution. The tissues were dehydrated in an ethanol series and were embedded in araldite via propylene oxide, with a three day penetration period. Sections were cut on a Porter-Blum hand operated microtome and stained for 45 minutes in a saturated solution of uranyl acetate in 50% ethanol, and for 20 minutes in lead citrate (Reynolds 1963).

Study of the surface view of the sperm tail shows it to consist of two longitudinally orientated portions, one of which shows strands of substructure also orientated longitudinally (fig 1). The sperm tail cut in transverse section also shows two principal elements (fig 2), one of which is more or less homogeneous in appearance while the other shows radially arranged substructure. These two regions are respectively the nebenkern and the axial region (Baccetti and Bairati, 1965). The mitochondria in the spermatid become re-organized into a single body which becomes the nebenkern (Yasuzumi, Fujimura and Ishida 1958). In some species, e.g. Dacus (Baccetti and Bairati 1965), this forms two nebenkern bodies of equal size, but in D. melanogaster there is one large nebenkern and one small one (fig. 2). The axial filament has a flagellar organization (Baccetti and Bairati 1965). Thus it is clear that the sperm tail consists of two principal elements: the large nebenkern and the axial region. It was also noted that these two may become separated (fig. 3).

It has been suggested (Oster et al 1966) that sperm tails split into two longitudinal strands by acetic acid treatment may be fragmented into the two regions here described. Of this there can be little doubt and it seems likely that in the present case the separation is due to the action of the fixative. Oster et al describe a number of reagents which cause the separation and a number which do not, but the principal criterion appears to be the osmotic potential of the reagent. It has been shown (Ballowitz 1890) that treatment with hypertonic solutions of osmic acid split beetle sperm tails into a number of separate fibers. Oster et al state that one of the two fibers is spiralized and is thicker than the other at the tail end of the sperm. It has been shown (Baccetti and Bairati 1965) that the nebenkern is much reduced in diameter towards the tail of the sperm while the axial region is less so. It would therefore seem likely that the 'spiralized fiber' of Oster et al is in fact the axial region and the 'straight fiber' is the nebenkern. The separation described and figured by Oster et al (see their figure 1B) would occur if the nebenkern decreased in size, particularly in the long axis, due to osmotic stress. If osmotic stress by a hypertonic bathing solution did exist then the nebenkern would be shortened longitudinally and the axial region, with its many internal struts, might resist this. The differential stress set up between the nebenkern and the axial region would cause them to separate at numerous points along their length, as described by Oster et al, and the subsequent shortening of the nebenkern would leave the axial region thrown into lateral folds. This seems a reasonable interpretation of figure 1B of Oster et al and is supported by observations of thin sections of sperm (figs. 4a and 4b) which show lengths of nebenkern cut longitudinally and axial region apparently passing in and out of the plane of the section.

Incidental to the study of sperm tail structure it was noted that the wall of individual coils of the ventral receptacle has an outer muscle coat (fig. 5). This muscle tissue is



Electron micrographs of sperm and the ventral receptacle

Fig. 1. Shadowed replica of part of sperm tail showing region of nebenkern (n) and axial region (a). Scale line = 1.0u.

Fig. 2. T.S. of sperm tail from spermatheca showing the axial region (a), the large nebenkern (n) and the small nebenkern (n_1). Scale line = 0.1u.

Fig. 3. Part of section of spermatheca with sperm tails in lumen cut in T.S. In most cases the two regions of the sperm tail have become separated (⟶). Scale line 0.5u.

Figs. 4a and 4b. L.S. of sperm tail showing axial region and nebenkern. The axial region passes in and out of the plane of the section. Scale line = 0.5u.

Fig. 5. T.S. of ventral receptacle showing sperm (sp) in lumen (l) of receptacle, cuticular lining (cu), cells of receptacle wall (wc), basement membrane (bm) and external to this a layer of visceral muscle (vm). Scale line = 1.0u.

Fig. 6. Part of the ventral receptacle wall showing visceral muscle (vm) cut mainly in T.S. Each thick filament is surrounded by 12 thin filaments (⟶). Scale line = 0.5u.

seen to show (fig. 6) the thick and thin filaments of myosin and actin, respectively, typical of muscle. Close examination of the muscle cut in transverse section shows that each thick filament is surrounded by twelve thin filaments as shown in insect visceral muscle (Smith, Gupta and Smith 1966), as opposed to six thin filaments found in insect flight muscle and other 'skeletal' muscle (Smith 1961). It has been stated (Demerec 1950) that the coiled ventral receptacle lacks muscle fibers, but this investigation has shown that throughout its length there is a narrow but well developed layer of visceral muscle surrounding the tube. This may assist in the emission of stored sperm, and may allow temporal control of this process.

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Kuhn, D.T. Arizona State University, Tempe, Arizona. Another case of mass mutation.

A case of mass mutation was encountered in D. melanogaster at Arizona State University between September 1966 and October 1967. The mutations appeared in an Urbana laboratory strain heterozygous for In(3L)P, st. Many germ line and somatic mutations were observed during this one year period. Whole body mutations such as ebony, Minute, yellow^2 and white were encountered more than once. White eyed males were observed on four different occasions.

The mass mutation phenomenon disappeared just as rapidly as it had appeared. Spencer (1935) noted a similar disappearance of visible mutations during his eight year study in D. funebris and D. hydei. He found two mutating periods that were separated by a three year interval during which time not a single visible mutation was observed.

Three months prior to the disappearance of the mass mutation phenomenon, an attempt was made to gather quantitative data on the frequency of spontaneous sex-linked lethals produced in the strain showing the mass mutation. Samples were taken in July, August and September of 1967. A frequency of 0.51 percent (393 X-chromosomes tested) lethals was observed in July. During August the frequency was 0.48 percent (1032 X-chromosomes tested), while in September it dropped to 0.21 percent (935 X-chromosomes tested). The sudden decrease in frequency of sex-linked lethals from August to September paralleled the disappearance of all visible mutations. From September 1967 to the present no more visible mutations have been observed in this strain.

Even though the sample of X-chromosomes tested was small, it is very possible that the simultaneous disappearance of visible mutations and decrease in the frequency of sex-linked lethals were not coincidental. An inactivation or alteration of a gene by a virus-like particle (Mampell, 1946) could result in either a visible mutation (germ line or somatic) or a mutation that would be lethal to the organism. Therefore, it is suggested that this strain became infected with a virus-like particle that was responsible for high frequencies of visible and sex-linked lethal mutations. In September the postulated virus-like particle abandoned the strain and the mutation rates reverted to frequencies characteristic for the strain.

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